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<120> METHODS AND COMPOSITIONS FOR USE IN
SPLICOSOME MEDIATED RNA TRANS-SPLICING

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<140> 09/756,095

<141> 2001-01-08

<150> 09/158,863

<151> 1998-09-23

<150> 09/133,717

<151> 1998-08-13

<150> 09/087,233

<151> 1998-05-28

<150> 08/766,354

<151> 1996-12-13

<150> 60/008,317

<151> 1995-12-07

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120

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132

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29

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36

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68

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35

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18

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51

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17

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gttctgtcct tgtctc
16

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<400> 20
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36

<210> 21
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catcgtcata atttccttgt g
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<210> 23
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<400> 25
accggaattc atgaagccag gtacaccagg
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gggcaagggtg aacgtggatg
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<210> 27
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<400> 27
atcaggagtg gacagatcc
19

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Escherichia coli lacZ gene

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39

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Escherichia coli lacZ gene

<400> 29
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36

<210> 30
<211> 38
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<220>
<223> Oligonucleotide primers complimentary to the
Escherichia coli lacZ gene

<400> 30
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38

<210> 31
<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primers complimentary to the
Escherichia coli lacZ gene

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38

<210> 32
<211> 47
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primers complimentary to the
Escherichia coli lacZ gene

<400> 32
gcatggtaac cctgcagggc ggcttcgtct aataatggga ctgggtg
47

<210> 33
<211> 37
<212> DNA
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<220>
<223> Oligonucleotide primers complimentary to the beta
HCG6 gene (accession #X00266)

<400> 33
gcatggatcc tccggagggc ccctggcac cttccac
37

<210> 34
<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primers complimentary to the beta
HCG6 gene (accession #X00266)

<400> 34
ctgactgcag ggtaaccgga caaggacact gttcacc
38

<210> 35
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primers complimentary to the beta
HCG6 gene (accession #X00266)

<400> 35
gcatggtaac cctgcagggg ctgctgctgt tgctg
35

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HCG6 gene (accession #X00266)

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ctgaaagctt gttaaccagc tcaccatggt gggcag
37

<210> 37
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<212> DNA
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<220>
<223> Oligonucleotide primers complimentary to the
Escherichia coli lacZ gene

<400> 37
ggcttcgct acctggagag ac
22

<210> 38

<211> 21
<212> DNA
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<220>
<223> Oligonucleotide primers complimentary to the
Escherichia coli lacZ gene

<400> 38
gctggatgcg gcgtgcggtc g
21

<210> 39
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Escherichia coli lacZ gene (accession #X00266)

<400> 39
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<212> DNA
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<400> 40
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45

<210> 41
<211> 35
<212> DNA
<213> Homo sapiens

<400> 41
acctctgcag gtgaccctgc aggaaaaaaaaa agaag
35

<210> 42
<211> 30
<212> DNA
<213> Homo sapiens

<400> 42
acctctgcag acttcacttc taatgatgtat
30

<210> 43
<211> 51
<212> DNA
<213> Homo sapiens

<400> 43
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51

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<211> 32
<212> DNA
<213> Homo sapiens

<400> 44
gacctctcga gggatttggg gaattatattt ag
32

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<211> 35
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<400> 45
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35

<210> 46
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<400> 46
ctgacacctcg gcccgtacatc tatctttttt atgtg
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<210> 47
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<212> DNA
<213> Homo sapiens

<400> 47
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32

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<400> 48
ctaatgatga tgatgatgat g
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<210> 49
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<400> 49
cgccataatga tgatgatgat g
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<210> 50
<211> 21
<212> DNA
<213> Homo sapiens

<400> 50
cttcttggtta ctcctgtcct g
21

<210> 51
<211> 32
<212> DNA
<213> Homo sapiens

<400> 51
gacctctcga gggatttggg gaattatgg ag
32

<210> 52
<211> 21
<212> DNA
<213> Homo sapiens

<400> 52
aactagaagg cacagtcgag g
21

<210> 53
<211> 24

<212> DNA
<213> Artificial Sequence

<220>
<223> trans-spliced product containing Human chorionic
gonadotropin gene 6 sequences and Corynebacterium
diphtheriae diphtheria toxin A sequence

<400> 53
gagatgttcc agggcgtgat gatg
24

<210> 54
<211> 125
<212> RNA
<213> Artificial Sequence

<220>
<223> PTM intramolecular base-paired stem

<221> misc_feature
<222> (57)...(70)
<223> Loop comprising a combination of 14 nucleotides
according to the specification

<400> 54
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60
nnnnnnnnnnn aucguuaacu aauaaacuac uaacuaacug ggugaauguu uuuucucggc
120
ugcag
125

<210> 55
<211> 127
<212> RNA
<213> Artificial Sequence

<220>
<223> PTM intramolecular base-paired stem

<221> misc_feature
<222> (57)...(70)
<223> Loop comprising a combination of 14 nucleotides
according to the specification

<400> 55
gcuagccugg gacaaggaca cugcuucacc cgguuaguag accacagccc ugagccnnnn

60

nnnnnnnnnnn aucguuaacu aauaaacuac uaacuggug aacuucugua uuauucucga
120
gcugcag
127

<210> 56
<211> 127
<212> RNA
<213> Artificial Sequence

<220>
<223> PTM intramolecular base-paired stem

<221> misc_feature
<222> (57) ... (70)
<223> Loop comprising a combination of 14 nucleotides
according to the specification

<400> 56
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60
nnnnnnnnnnn aucguuaacu aauaaacuac uaacuggug aaguucuguc cuugucucga
120
gcugcag
127

<210> 57
<211> 132
<212> DNA
<213> Artificial Sequence

<220>
<223> trans-spliced product containing Human chorionic
gonadotropin gene 6 sequences and Corynebacterium
diphtheriae diphtheris toxin A sequences

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caggggacgc accaaggatg gagatgttcc agggcgctga tgatgttgtt gattcttctt
60
aaatcttttg tgatggaaaaa ctttcttcg taccacggga ctaaacctgg ttatgttagat
120
tccattcaaa aa
132

<210> 58
<211> 18
<212> DNA

<213> Artificial Sequence

<220>

<223> Artificial sequence comprising sequences derived from Escherichia coli lacZ gene

<400> 58
gaattcggta ccatgggg
18

<210> 59
<211> 33
<212> DNA
<213> Artificial Sequence

<220>

<223> Artificial sequence comprising sequences derived from Escherichia coli lacZ gene and

<400> 59
cgtttacagg taagaggatc ctccggaggg ccc
33

<210> 60
<211> 30
<212> DNA
<213> Artificial Sequence

<220>

<223> Artificial sequence comprising sequences derived from Escherichia coli lacZ gene

<400> 60
tggtgtcaaa aataataagt taacaagctt
30

<210> 61
<211> 25
<212> DNA
<213> Artificial Sequence

<220>

<223> trans-spliced product containing Escherichia coli lacZ gene sequences and Human chorionic gonadotropin gene 6 exon 2 sequences

<400> 61
cagcagcccc tgtaaacggg gatac

25

<210> 62
<211> 286
<212> DNA
<213> Artificial Sequence

<220>
<223> trans-spliced product containing Escherichia coli
lacZ gene sequences

<400> 62
ggcttcgct acctggagag acgcgcgc tgatccttg cgaatacgcc cacgcgttgg
60
gtaacagtct tggcggtttc gctaaataact ggcaggcggt tcgtcagtat ccccgtttac
120
agggcggctt cgtctaataa tggactggg tggatcagtc gctgattaaa tatgtatgaaa
180
acgggcaacc cgtggtcggc ttacggcggt gatttggcg atacgccgaa cgatcgccag
240
ttctgtatga acggctcggt ctggccgac cgcacgcccgc atccag
286

<210> 63
<211> 196
<212> DNA
<213> Artificial Sequence

<220>
<223> trans-spliced product containing Escherichia coli
lacZ gene sequences

<400> 63
ggcttcgct acctggagag acgcgcgc tgatccttg cgaatacgcc cacgcgttgg
60
gtaacagtct tggcggtttc gctaaataact ggcaggcggt tcgtcagtat ccccgtttac
120
aggggctgct gctgttgctg ctgctgagca tggcgccgac atggcatcc aaggagccac
180
ttcggccacg gtggcg
196

<210> 64
<211> 420
<212> DNA
<213> Artificial Sequence

<220>

<223> trans spliced product comprising cystic fibrosis transmembrane regulator-derived sequences and His tag sequences

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aacgttgctc gagtactaac tggAACCTCT tctttttt cctgcagact tcacttctaa
120
tcatgttattt gggagaactg gagccttcag agggtaaaat taagcacagt ggaagaattt
180
cattctgttc tcagtttcc tggattatgc ctggcaccat taaagaaaat atcatcttg
240
gcggccgcca ctgtgctgga tatctgcaga attccaccac actggactag tggatccgag
300
ctcgg tacca aggttaagtt taaaccgctg atcagcctcg actgtgcctt ctagttgcca
360
gccatctgtt gtttgcctt cccccgtgcc ttccttgacc ctggaaggta ccactccac
420

<210> 65

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Splice junction sequence

<400> 65

atgttccagg gcgtgatgat

20

<210> 66

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> C terminal residues of glutathione-S-transferase

<400> 66

Asp Tyr Lys Asp Asp Asp Lys

1

5

<210> 67

<211> 15

<212> DNA
<213> Artificial Sequence

<220>
<223> Artificial Sequence comprising sequences derived
from Escherichia coli lacZ gene

<400> 67
ggagttgatc ccgtc
15

<210> 68
<211> 37
<212> DNA
<213> Artificial Sequence

<220>
<223> Artificial Sequence comprising sequences derived
from Escherichia coli lacZ gene and human
chorionic gonadotropin gene 6 intron 1

<400> 68
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37

<210> 69
<211> 120
<212> DNA
<213> Artificial Sequence

<220>
<223> Binding domain of PTM

<400> 69
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60
tattaactca tttgattcaa aatatttaaa atacttcctg tttcatactc tgctatgcac
120

<210> 70
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Spacer sequences of PTM

<400> 70
aacattatta taacgttgct cgaa
24

<210> 71
<211> 47
<212> DNA
<213> Artificial Sequence

<220>
<223> Branch point, pyrimidine tract and acceptor splice site of PTM

<400> 71
tactaactgg taccttttct ttttttttg atatcctgca gggcggc
47

<210> 72
<211> 70
<212> DNA
<213> Artificial Sequence

<220>
<223> Donor site and spacer sequence of PTM

<400> 72
tgaacggtaa gtgttatcac cgatatgtgt ctaacctgat tcgggccttc gatacgctaa
60
gatccaccgg
70

<210> 73
<211> 260
<212> DNA
<213> Artificial Sequence

<220>
<223> Binding domain of spacer sequence

<400> 73
tcaaaaagtt ttacataat ttcttacctc ttcttgaatt catgcttga tgacgcttct
60
gtatctatat tcattttttttttaatggcc tggcataatc
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ctggaaaact gataacacaa tgaaattctt ccactgtgct taaaaaaacc ctcttgaatt
180
ctccatttct cccataatca tcattacaac tgaactctgg aaataaaacc catcattatt
240

aactcattat caaatcacgc
260

<210> 74
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer

<400> 74
cgctggaaaa acgagcttgt tg
22

<210> 75
<211> 23
<212> DNA
<213> Oligonucleotide Artificial Sequence

<220>
<223> Oligonucleotide primer

<400> 75
actcagtgtg attccacacctt ctc
23

<210> 76
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide

<400> 76
gacctctgca gacttcactt ctaatgatga ttatgg
36

<210> 77
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer

<400> 77

ctaggatccc gttctttgt tcttcactat taa
33

<210> 78
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer

<400> 78
ctagggttac cgaagtaaaaa ccatacttat tag
33

<210> 79
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer

<400> 79
gcatggttac cctgcagggg ctgctgctgt tgctg
35

<210> 80
<211> 37
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer

<400> 80
ctgaaagctt gttaaccagc tcaccatggt gggcag
37

<210> 81
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Binding domain of PTM molecule

<400> 81

accatcatt attaggtcat tat
23

<210> 82
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer

<400> 82
gatcaaatct gtcgatcctt cc
22

<210> 83
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer

<400> 83
ctgatccacc cagtcccatt a
21

<210> 84
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer

<400> 84
gactgatcca cccagtccta ga
22

<210> 85
<211> 52
<212> DNA
<213> Artificial Sequence

<220>
<223> Random sequences inserted to replace 3' splice
site

<221> misc_feature
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<400> 85
ccgcggnnnn nnnnnnnnnn nnnnnnnnnn gggttccgggt accggcggct tc
52

<210> 86
<211> 71
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide

<400> 86
tttatcccc gtttacaggg cggcttcgtc tggactggg tggatcagtc gctgattaaa
60
tatgatgaaa a
71

<210> 87
<211> 66
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide

<400> 87
tttggcgata cgccgaacga tcgccagttc tgtatgaacg gtctggtctt tgccgaccgc
60
acgccc
66

<210> 88
<211> 192
<212> DNA
<213> Artificial Sequence

<220>
<223> PTM sequences

<400> 88
acgagcttgc tcatgatgat catgggcgag ttagaaccaa gtgaaggcaa gatcaaacat
60

tccggccgca tcagctttg cagccaaattc agttggatca tgcccggtac catcaaggag
120
aacataatct tcggcgtag ttacgacgag taccgctatc gctcggtgat taaggcctgt
180
cagttggagg ag
192

<210> 89
<211> 25
<212> DNA
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<220>
<223> Oligonucleotide

<400> 89
gagcaggcaa gacgagcttg ctcat
25

<210> 90
<211> 28
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<220>
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<400> 90
gagaacataaa tcttcggcgt cagttacg
28

<210> 91
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<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide

<400> 91
gtcagttgga ggaggacatc tccaagttt
30

<210> 92
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<212> DNA
<213> Artificial Sequence

<220>
<223> PTM sequences

<400> 92
acgagcttgc tcatgatgat catggcgag ttagaaccaa gtgaaggcaa gatcaaacat
60
tccggccgca tcagctttg cagccaattc agttggatca tgcccggtac catcaaggag
120
aacataaatct tcggcgtagt ttacgacgag taccgctatc gctcggtgat taaggcctgt
180
cagttggagg ag
192

<210> 93
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<212> DNA
<213> Artificial Sequence

<220>
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<400> 93
aaatatcatt ggtgtttctt atgatga
27

<210> 94
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<220>
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<400> 94
ccaactagaa gaggacatct ccaagttgc
30

<210> 95
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<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide

<400> 95
atgatcatgg gcgagttaga accaagttag
30

<210> 96
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide

<400> 96
aaaatatcat cttgggtgtt tcctatg
27

<210> 97
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide

<400> 97
ccaaactagaa gaggacatct ccaagtt
27

<210> 98
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> 5' splice site

<400> 98
cgtttacagg taagtggatc c
21

<210> 99
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> 3' splice site

<400> 99
ctgcagggcg gcttcgtcta ataatgg
27

<210> 100
<211> 46
<212> DNA
<213> Artificial Sequence

<220>
<223> Sequence from trans-splicing domain

<400> 100
tactaactgg tacctcttct tttttttga tatcctgcag ggccgc
46

<210> 101
<211> 1584
<212> DNA
<213> Artificial Sequence

<220>
<223> CFTR PTM

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120
ccttctgttg attctgctga caatcttatct gaaaaattgg aaagagaatg ggatagagag
180
ctggcttcaa agaaaaatcc taaactcatt aatgcccttc ggcgatgttt tttctggaga
240
tttatgttct atggaatctt tttatattta ggggaagtca ccaaagcagt acagcctctc
300
ttactggaa gaatcatagc ttccatgac ccggataaca aggaggaacg ctctatcgcg
360
atttatctag gcataggctt atgccttctc tttattgtga ggacactgct cctacaccca
420
gccatTTTG gcTTTcatca cattggaatg cagatgagaa tagctatgtt tagTTGATT
480
tataagaaga cttaaaagct gtcaagccgt gttctagata aaataagtat tggacaactt
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gttagtctcc ttTCCAACAA CCTGAACAAA TTTGATGAAG GACTTCATT GGCACATTG
600
gtgtggatcg ctccttgca agtggcactc ctcatggggc taatctggga gttgttacag
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gcgtctgcct tctgtggact tggTTTCTG atagtccttg cccttttca ggctgggcta
720
gggagaatga tcatgttggactt cagagatcag agagctggga agatcagtga aagacttgc
780

attacctcag aaatgatcga gaacatccaa tctgttaagg catactgctg ggaagaagca
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1260
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1320
ctgaaagata ttaatttcaa gatagaaaga ggacagttgt tggcggttgc tggatccact
1380
ggagcaggca agacgagctt gctcatgatg atcatggcg agttagaacc aagtgaaggc
1440
aagatcaaac attccggcccg catcagctt tgcagccaat tcagttggat catgcccggt
1500
accatcaagg agaacataat cttcggcgac agttacgacg agtaccgcta tcgctcggtg
1560
attaaggcct gtcagttgga ggag
1584

<210> 102

<211> 323

<212> DNA

<213> Artificial Sequence

<220>

<223> Trans-splicing domain of CFTR PTM

<400> 102

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ggtaaaaaag tttcacata atttcttacc tcttcttgc ttcatgcttt gatgacgctt
120
ctgtatctat attcatcatt ggaaacacca atgatatttt cttaatggt gcctggcata
180
atccctggaaa actgataaca caatgaaatt cttccactgt gcttaatttt accctctgaa
240
ttctccattt ctcccataat catcattaca actgaactct ggaaataaaaa cccatcatta
300

ttaactcatt atcaaatcac gct
323

<210> 103
<211> 165
<212> DNA
<213> Artificial Sequence

<220>
<223> PTM binding domain

<400> 103
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cctaaggaga agtgttatatt ctatattgt aagattctat taactcattt gattcaaaat
120
attnaaaata cttcctgttt cacctactct gctatgcacc cgccgg
165

<210> 104
<211> 225
<212> DNA
<213> Artificial Sequence

<220>
<223> Trans-splicing domain of CFTR PTM

<400> 104
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gcagaagtgt atattcttat ttgtaaagat tctattaact catttgcattt aaaatattta
120
aaataacttcc tgttcacct actctgctat gcacccgcgg aacattatta taacgttgct
180
cgaataactaa ctggcaccc tc ttctttttt tttgatatcc tgcag
225

<210> 105
<211> 3069
<212> DNA
<213> Artificial Sequence

<220>
<223> CFTR PTM sequence

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60

agtggaaagaa tttcattctg ttctcagttt tcctggatta tgcctggcac cattaaagaa
120
aatatcatct ttgggtgttc ctatgatgaa tatagataca gaagcgtcat caaagcatgc
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caactagaag aggacatctc caagttgca gagaaagaca atatagttct tggagaaggt
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300
gatgctgatt tgtatttatt agactctcct tttggatacc tagatgtttt aacagaaaaaa
360
gaaatatttg aaagctgtgt ctgtaaactg atggctaaca aaactaggat tttggtcact
420
tctaaaatgg aacatttaaa gaaagctgac aaaatattaa ttttgcata agtagcagc
480
tatTTTATG ggacattttc agaactccaa aatctacagc cagacttag ctcAAAactc
540
atgggatgtg attcttcga ccaatttagt gcagaaagaa gaaattcaat cctaactgag
600
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660
caatcttta aacagactgg agagttggg gaaaaaagga agaattctat tctcaatcca
720
atcaactcta tacgaaaatt ttccattgtg caaaagactc ctttacaaat gaatggcatc
780
gaagaggatt ctgatgagcc tttagagaga aggctgtcct tagtaccaga ttctgagcag
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3069